Systems Pharmacology PHAR3320

Nerves of the Respiratory Tract

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Learning Outcomes

- By the end of this lecture, students should be able to
  - describe nerve pathways that innervate the airways
  - discuss neuromodulation of cholinergic pathways
  - describe the potential effects of eosinophils, $M_2$ muscarinic receptor dysfunction and rho-kinase on cholinergic nerve function in the airways

Cholinergic and Adrenergic Nerves

- Excitatory=contraction  Inhibitory=relaxation
- Cholinergic (excitatory)
  - acetylcholine
  - dominant respiratory neural pathway
    - all levels of the respiratory tract
    - airway smooth muscle tone and mucus production
- Noradrenergic (inhibitory)
  - noradrenaline
  - no dominant role below main bronchi (human)
  - innervates cholinergic ganglia, not airway smooth muscle directly (human)
  - $\beta_2$-receptors in airway smooth muscle
**Excitatory Nonadrenergic Noncholinergic**

- eNANC neurotransmitters = sensory neuropeptides
  - substance P, neurokinin A, calcitonin gene-related peptide (CGRP)
- Stimulation of eNANC nerves ↑ airway tone, secretion of mucus, microvascular leakage
- Inhibition of eNANC nerves
  - neurokinin receptor antagonists
  - nociceptin ↓ neuropeptide release with EFS (Shah et al., 1998 Br J Pharmacol, 123, 510-516)
- eNANC nerves may be “stimulated” or “enhanced”
  - capsaicin
  - neutral endopeptidase inhibitors
    - thiorphan, phosphoramidon

**Inhibitory Nonadrenergic Noncholinergic**

- Nitric oxide
  - L-arginine + NADPH + O₂ → NO + citrulline + NADP
  - nitro-L-arginine methyl ester (L-NAME, nitric oxide synthase inhibitor) inhibits nitric oxide synthase (NOS)
  - L-NAME inhibits synthesis of NO
- Nitric oxide activates guanylyl cyclase
  - cGMP, relaxation
- may ↓ secretion of mucus and ↓ microvascular permeability
Modulation of Cholinergic Nerve Function

- **Noradrenaline**, **acetylcholine**, **substance P**, **histamine**, **prostaglandin E₂**, **sarafotoxin S6c**
- Agonist: **acetylcholine**
- **Postganglionic cholinergic nerve**
- **M₃** muscarinic receptor
- **Rho-kinase pathway**
- **Airway smooth muscle cell**
- **Acetylcholine agonist**
- **Contraction**

Eosinophils

- Asthma characterised by the presence of inflammatory cells eg eosinophils
  - ↑ BAL fluid, induced sputum, bronchial wall biopsies
  - Histological examination of tissue from fatal asthmatics
    • ↑ activated, degranulating eosinophils + toxic mediators
- Eosinophils release mediators that mimic asthma
  - Toxic cationic proteins eg major basic protein (MBP)
  - Lipid molecules eg platelet activating factor (PAF)
  - Cytokines that activate other inflammatory cells eg TNF-α

Acetylcholine: Synthesis, Storage and Release

- Acetylcholine synthesised from choline and acetyl CoA via **choline acetyltransferase** (ChAT)
- ACh transported into synaptic vesicles by the vesicular acetylcholine transporter (VACHT)
- Acetylcholine activates pre- (M₂) and post-synaptic (M₃) muscarinic receptors
- Acetylcholine metabolised into choline and acetate via **acetylcholinesterase** (AChE)
- Enzymes and receptors control ACh synthesis, uptake, activity and turnover in cholinergic nerves

Modulation of Cholinergic Responses

- Dominant neural input to the respiratory tract
- Enhanced cholinergic nerve function
  - Eosinophils
  - M₂ muscarinic receptor dysfunction
  - Rho-kinase pathway
- Augmentation of cholinergic nerve function may contribute to ↑ airway smooth muscle tone and secretion of mucus
- Some examples of cholinergic nerve modulation
Eosinophils and Cholinergic Nerves

- Eosinophils accumulate at cholinergic nerves in human and animal allergic models
  - asthma, rhinitis, eosinophilic gastroenteritis, inflammatory bowel disease
- Interaction between eosinophils and nerves has been shown to cause cholinergic nerve remodelling (Durcan et al. 2006 Am J Respir Cell Mol Biol, 34, 775-786)
- Eosinophil adhesion to nerves stimulates the release of eosinophil-derived factors
- Eosinophils contain nerve growth factor (NGF), major basic protein (MBP), eosinophil-derived neurotoxin (EDN), eosinophil peroxidase (EPO), eosinophil cationic protein (ECP)
  - modulate cholinergic nerve function?

Eosinophils: Cholinergic Nerve Remodelling

- Co-incubation of eosinophils + cholinergic nerve-like cells (Durcan et al., 2006)
  - induced ChAT mRNA + protein production
  - induced VACHT mRNA + protein production
  - packaging of newly synthesised ACh?
  - ACh release from synaptic vesicles?
  - acetylcholine content
  - suggests synthesis and uptake into vesicles
    - intracellular levels?
  - choline content
  - choline uptake for ACh synthesis?
  - acetylcholinesterase activity
  - prolonged action at the synapse?

M₂ Receptor Function

- M₂ receptor on postganglionic cholinergic nerve
  - acetylcholine
  - negative feedback
  - M₂
  - cholinergic synapse
  - airway smooth muscle cell
  - contraction

M₂ Receptor Dysfunction

- M₂ receptor on postganglionic cholinergic nerve
  - negative feedback
  - acetylcholine
  - M₂
  - cholinergic synapse
  - airway smooth muscle cell
  - contraction
Neuronal M$_2$ Receptor Dysfunction

- ↑ acetylcholine release
  - antigen challenge, virus infection, ozone exposure, Vitamin A deficiency
- Eosinophils associated airway nerves in asthma, allergy
  - major basic protein (MBP) inhibits M$_2$ receptor function
  - co-incubation of eosinophils with cholinergic nerve-like cells (Durcan et al., 2006) results in adhesion and modulation of neuronal M$_2$ receptor expression
    - ↑ M$_2$ receptor expression
      - nerve growth factor, major basic protein, eosinophil peroxidase
      - combination of major basic protein, eosinophil peroxidase and eosinophil-derived neurotoxin
    - ↓ M$_2$ receptor expression
      - eosinophil-derived neurotoxin

Obesity and Hyperinsulinemia

- Obesity is a risk factor for asthma
  - In obese-prone and obese-resistant rats (Nie et al. 2014 Am J Respir Cell Mol Biol, 51, 251-261)
    - ↑ vagally-mediated bronchoconstriction in obese rats on high fat diet
    - M$_2$ receptor-mediated inhibition of vagally-induced bronchoconstriction ↓ in obese-prone rats
    - bronchoconstriction to ACh unaltered – implication?
    - obesity did not induce airway inflammation or smooth muscle hypertrophy
    - ↓ serum insulin protected neuronal M$_2$ muscarinic receptor function
    - insulin ↓ M$_2$ muscarinic receptor function in human airway preparations
- Effectiveness of anticholinergic drugs in this phenotype?

Glucocorticoids and Neuronal M$_2$ Receptors

- Glucocorticoids frontline anti-inflammatory therapy in asthma
- Experimentally, glucocorticoids
  - ↑ M$_2$ receptor gene expression
  - ↑ M$_2$ receptor functional activity
  - protect M$_2$ receptor function
  - ↑ degradation of acetylcholine by cholinesterases
  - prevent eosinophil recruitment to airway nerves
- Additional mechanisms of action with respect to therapy

Airway Smooth Muscle Contraction

- agonist
  - Ca$^{2+}$
  - voltage-gated channel
  - phospholipase C
    - inositol trisphosphate
  - myosin light chain kinase
    - Ca$^{2+}$-calmodulin
    - relaxation
  - myosin phosphatase
  - contraction
  - MLC-P
  - sarcoplasmic reticulum

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Smooth Muscle Contraction: *Rho-kinase*

- agonist
- Rho-GTP
- Rho-kinase
- phospholipase C
- inositol trisphosphate
- Ca²⁺
- voltage-gated channel
- Ca²⁺-calmodulin
- myosin light chain kinase
- relaxation
- MLC
- MLC-P
- contraction
- myosin phosphatase
- sarcoplasmic reticulum

*Rho-kinase* Inhibitors in the Airways

- *Rho/Rho-kinase* is involved in a range of biological functions initiated following activation of G protein-coupled receptors
- *Rho-kinase* inhibitor exhibits bronchodilator as well as anti-inflammatory activity (Henry et al. 2005 Pulm Pharmacol Ther, 18, 67-74)

*Rho-kinase* Inhibitor vs Cholinergic Contractile Responses

- postganglionic cholinergic nerve
- cholinergic synapse
- acetylcholine
- M₃
- contraction

Cholinergic Nerve-Mediated Contraction

- force transducer
- platinum electrode
- propranolol (1 μM)
- L-NAME (100 μM)
- indomethacin (3 μM)
- 3Hz, 3v, <200mA, 0.5ms, 10s
**Acetylcholine Release from Cholinergic Nerves**

- Airways exposed to [H]-choline and electrically stimulated (EFS)
- Efflux of radioactivity derived from [H]-choline measured by liquid scintillation counting
- Two periods of EFS (S1 and S2)
- Effect of various drugs on neurotransmitter release from cholinergic nerves assessed
  - Ratio of S2:S1 (area under the curve, AUC)

**Effect of Tetrodotoxin on ACh Release**

- Radioactivity released (dpm/g/3 min)
- Control
- TTX

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**Rho-kinase Inhibitor vs Nerve-Mediated Contraction**

- 50 μM Rho-kinase inhibitor
- Time control
- 13v, 0.5ms, 10s, <200mA, 3Hz

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**Rho-kinase Inhibitor vs Nerve-Mediated Contraction**

- *Rho-kinase* inhibitor ↓ cholinergic nerve-mediated contraction
- *Rho-kinase* inhibitor ↑ acetylcholine release
- Inhibitory effect of *Rho-kinase* inhibitor due to antagonism of acetylcholine-induced contraction (Fernandes et al., 2006, Eur. J. Pharmacol., 550, 155-161)
  - inhibitory effect on airway smooth muscle
  - overcomes ↑ neurotransmitter release

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**Nerves of the Respiratory Tract Readiness Assurance Test (RAT)**

- Readiness Assurance Test (40%)
  - 1 – 1.30pm
  - extended matching questions, MCQs, SAQ and drug dilution calculations
  - bring a calculator
  - online
  - material presented in the laboratory handout and the Nerves of the Respiratory tract lecture
- Lab report (60%)